

singly used in antitumoral therapies and mTOR inhibition with rapamycin was shown to be cardioprotective during aging and cardiac stress. Studies in genetic mice models have shown that mTOR is essential for heart development and cardiac function in adult. However, mTOR functions during postnatal cardiac development are not fully elucidated. We have therefore generated a cardiac-specific mTOR knockout mouse using α -MHC-Cre mice leading to mTOR inactivation in early postnatal mouse myocardium. The mutant mice develop a severe lethal dilated cardiomyopathy due to defects in cardiomyocyte growth, survival and subsequent fibrosis. In contrast to adult myocardium, both mTORC1 and mTORC2 activities are impaired in juvenile heart, as shown by hypophosphorylation of the translation inhibitor 4E-BP1 and loss of the cardioprotective AKTS473 phosphorylation. We find that translation initiation defects and altered ribosome biogenesis both contribute to impaired cardiomyocyte growth. In addition, we show that increased apoptosis is associated with activation of JNK kinase and p53 accumulation. Moreover mTORcmKO hearts display a strong decreased expression of the primary oxygen carrier, myoglobin, and HIF1 α accumulation suggesting hypoxia. However, mTORcmKO hearts do not display HIF1 hypoxic response consistently with mTOR being essential for HIF1-dependant transcriptional activity. These observations indicate that hypoxia-induced apoptosis likely contribute to DCM in mTORcmKO mice. Altogether, our results demonstrate that mTOR is a key regulator of cardiomyocyte growth, viability and oxygen supply in early postnatal myocardium. Our findings highlight potential cardiotoxicity of new mTOR inhibitors and the importance to set up optimal treatments in cardiology to both target mTOR hypertrophic functions and maintain adequate oxygen supply.

0406

Effects of FGF23 and Klotho on adult rat cardiomyocytes in culture

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The bone derived hormone fibroblast growth factor 23 (FGF23) and its co-receptor Klotho represent a novel endocrine axis regulating mineral metabolism in health and disease.

FGF23-Klotho signalling inhibits renal phosphate reabsorption and activation of vitamin D, and reduces secretion of parathyroid hormone. Serum levels of FGF23 rise in chronic kidney disease (CKD). In contrast, tissue expression of Klotho decreases in parallel with CKD progression and reaches low or undetectable levels in end stage renal disease.

Numerous studies identify elevated FGF23 as a predictor of adverse clinical outcome. In particular, elevated FGF23 has recently been associated with greater risks of major cardiovascular events and mortality.

However, there have been very few studies that have attempted to address the direct effects of FGF23 on myocardium. Moreover whether Klotho is involved in FGF23 – mediated actions on cardiomyocytes is still unclear.

In this context, we investigate the role of FGF23 and Klotho in adult rat ventricular myocytes (ARVMs). Using video-edge-detection, epifluorescent microscopy and an Ionoptix® system, performed in isolated cardiomyocytes subjected to FGF23 or Klotho alone, or in association, we showed that FGF23 increases cell size and cell shortening in ARVMs, and induces arrhythmia in the presence of Isoprenaline. In addition Klotho prevents FGF23 effects on adult cardiomyocytes. Indeed, ARVMs subjected to Klotho showed marked protection from FGF23-induced hypertrophic responses and from FGF23-induced arrhythmias in the presence of Isoprenaline.

Altogether these preliminary data provide a direct evidence of the role FGF23 in adult cardiomyocytes and suggest that Klotho may have a beneficial effect in preventing adverse cardiovascular outcomes in patients with or without CKD.

0379

Cytoarchitectural and metabolic alterations induced by ER stress in heart

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The rough endoplasmic reticulum (rER) is the site for synthesis, folding and quality control of secreted and membrane proteins. Impairment of ER function in response to stresses such as oxidative stress, disruption of calcium homeostasis or ischemia causes the accumulation of misfolded proteins in the rER lumen, resulting in ER stress. Over the past decade, ER stress has emerged as an important mechanism involved in the pathogenesis of cardiovascular diseases including heart failure. However, the molecular mechanisms underlying the contribution of ER stress to cardiac dysfunction remain poorly understood. In the present study, we evaluated the effect of the ER stressor tunicamycin (TN) on cardiac function in mice. TN injection (2mg/kg, 72h) induced a significant impairment of systolic function as indicated by the decrease in ejection fraction and fractional shortening. However, the heart rate, left ventricular internal diameters in diastole and systole and wall thickness were not affected. Transmission electron microscopy analysis revealed that TN induced an important ultrastructural remodeling of the cardiomyocytes with an increase in the occurrence of rER. Whereas rER was essentially located near the nucleus in cardiomyocytes of control mice, we observed an expansion of the rER network near sarcomeres and around T-tubules and mitochondrial clusters after TN treatment. In addition, mitochondrial structure and network were also disorganized. When measured in skinned fibers, the rate of mitochondrial oxidation was slower and an impairment of the function of the creatine kinase energy shuttle was observed in response to TN. In addition, ER stress triggered a metabolic remodeling characterized by a shift from fatty acid to glycolytic substrates consumption. Taken together our results show for the first time that the cytoarchitectural and metabolic alterations of cardiomyocytes contribute to the cardiac injury induced by ER stress.

0116

Phosphodiesterases regulate cAMP level in the mitochondria of adult cardiomyocytes

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The heart is highly energy-dependent with most of its energy provided by mitochondria. Mitochondria also play a role in many essential cellular processes including metabolite synthesis, redox balance, calcium homeostasis and cell death. Therefore, maintaining a functional population of mitochondria is critical for cardiac function and identification of novel regulatory mechanisms. Recently, a soluble adenyl cyclase (sAC) has been revealed to serve as a local source of the second messenger cAMP in the mitochondrial matrix in response to bicarbonate and calcium. PDE2A, a cGMP-stimulated cAMP-hydrolyzing phosphodiesterase (PDE), was also shown to be expressed in the mitochondrial matrix of liver and brain. To gain new insights into the control of mitochondrial pool of cAMP, we investigated the role of various isoforms of PDEs in isolated cardiac mitochondria. Western blot analysis of PDE protein expression revealed positive bands for PDE2A, 3A, 4A and 4B subtypes in cardiac mitochondria. Basal cAMP-degrading enzymatic activities were determined by radioenzymatic assay in cardiac mitochondrial lysates with 1 μ m cAMP as substrate. PDE2 represented the largest mitochondrial cAMP PDE activity (35% of total) and its activity was enhanced ~2-fold by 5 μ M cGMP and was inhibited by the PDE2 inhibitor, Bay 60-7550 (100nm). PDE3 and PDE4 activities represented, respectively, 30% and 25% of total, and they were inhibited by their respective inhibitors, cilostamide (1 μ m) and Ro 20-1724 (10 μ M). Accordingly, measurements by ELISA of mitochondrial cAMP levels confirmed that inhibition of PDE2, 3 and 4 families leads to an increase in cAMP level. In conclusion, at least three PDE families and four PDE subtypes are located in the cardiac mitochondrial matrix, participating to a local signaling pathway with sAC to control cAMP level. Our findings unravel a cAMP signaling cascade in cardiac mitochondria which may have implications for the metabolic control of cardiac function.